G-8176/ #18

PATENT
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NO. 61944

9-1-99 OW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Coppens

Serial No.: 08/898,736

Filed: July 23, 1997

Title: PROCESS FOR THE PREPARATION OF MALTED CEREALS

Group Art Unit: 1761

Examiner: Sherrer

CERTIFICATE OF MAILING

I hereby certify that this paper is being deposited with the United States Postal Service as first class mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington, D.C. 20231, on this date.

8/13/99 James P. Krueger
Date Registration No. 35,234
Attorney for Applicant(s)

TRANSMITTAL OF CERTIFIED COPY OF FOREIGN PRIORITY DOCUMENT

Honorable Commissioner of Patents
and Trademarks
ATTENTION: Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

Enclosed herewith is a certified copy of the foreign priority document, PCT/BE96/00077 filed 23 July 1996, which is submitted to comply with 35 U.S.C. §119, in respect of the above-identified application.

Respectfully submitted,

FITCH, EVEN, TABIN & FLANNERY

By James P. Krueger
James P. Krueger
Registration No. 35,234

Date: August 13, 1999

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KONINKRIJK BELGIË

MINISTERIE VAN ECONOMISCHE ZAKEN

BESTUUR HANDELSBELEID

Er wordt bevestigd dat de stukken in bijlage gelijkluidende afschriften zijn van de documenten die de Dienst voor Industriële Eigendom in bezit heeft.

Brussel, de 20. -7 - 1999

Voor de Adviseur van de Dienst
voor de Industriële Eigendom

De gemachtigde Ambtenaar,

P. LAURENT
ADJUNCT-ADVISEUR

CERTIFIED COPY OF
PRIORITY DOCUMENT

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

PCT/BE96/00077
International Application No.

23 JUIL 1996 (23-07-1996)
International Filing Date

RO/BE - PCT INTERNATIONAL APPLICATION
Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) P.KUL.01/WO

Box No. I TITLE OF INVENTION

PROCESS FOR THE PREPARATION OF MALTED CEREALS.

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

CARGILL FRANCE N.V.
dba CARGILL MALT DIVISION N.V.
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☐ This person is also inventor.

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Teleprinter No.

State *(i.e. country)* of nationality: FRENCH

State *(i.e. country)* of residence: BELGIUM

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

COPPENS Theo
Nieuwstraat, 37
B-3120 TREMELO
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This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BELGIAN

State *(i.e. country)* of residence: BELGIUM

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

VAN MALDEREN Eric
OFFICE VAN MALDEREN
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Telephone No. 02/426.38.10

Facsimile No. 02/426.37.60

Teleprinter No. 63628 patbel b

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

DELCOUR Jan
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- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BELGIAN

State *(i.e. country)* of residence: BELGIUM

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [X] the United States of America only
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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

ISERENTANT Dirk
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This person is:
- [ ] applicant only
- [X] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality: BELGIAN

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This person is applicant for the purposes of:
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Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
- [ ] the United States of America only
- [ ] the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

This person is:
- [ ] applicant only
- [ ] applicant and inventor
- [ ] inventor only *(If this check-box is marked, do not fill in below.)*

State *(i.e. country)* of nationality:

State *(i.e. country)* of residence:

This person is applicant for the purposes of:
- [ ] all designated States
- [ ] all designated States except the United States of America
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- [ ] Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- [X] AP **ARIPO Patent:** KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- [X] EA **Eurasian Patent:** AZ Azerbaijan, BY Belarus, KZ Kazakstan, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT KG Kirghizstan, MD Republic of Moldova, AM Armenia
- [X] EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, DE Germany, DK Denmark, ES Spain, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT FI Finland
- [X] OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- [X] AL Albania
- [ ] AM Armenia
- [ ] AT Austria
- [X] AU Australia
- [ ] AZ Azerbaijan
- [X] BB Barbados
- [X] BG Bulgaria
- [X] BR Brazil
- [ ] BY Belarus
- [X] CA Canada
- [ ] CH and LI Switzerland and Liechtenstein
- [X] CN China
- [X] CZ Czech Republic
- [ ] DE Germany
- [ ] DK Denmark
- [X] EE Estonia
- [ ] ES Spain
- [ ] FI Finland
- [ ] GB United Kingdom
- [X] GE Georgia
- [X] HU Hungary
- [X] IS Iceland
- [X] JP Japan
- [ ] KE Kenya
- [ ] KG Kyrgyzstan
- [X] KP Democratic People's Republic of Korea
- [X] KR Republic of Korea
- [ ] KZ Kazakstan
- [X] LK Sri Lanka
- [X] LR Liberia
- [ ] LS Lesotho
- [X] LT Lithuania
- [ ] LU Luxembourg
- [X] LV Latvia
- [ ] MD Republic of Moldova
- [X] MG Madagascar
- [X] MK The former Yugoslav Republic of Macedonia
- [X] MN Mongolia
- [ ] MW Malawi
- [X] MX Mexico
- [X] NO Norway
- [X] NZ New Zealand
- [X] PL Poland
- [ ] PT Portugal
- [X] RO Romania
- [ ] RU Russian Federation
- [ ] SD Sudan
- [ ] SE Sweden
- [X] SG Singapore
- [X] SI Slovenia
- [X] SK Slovakia
- [ ] TJ Tajikistan
- [ ] TM Turkmenistan
- [X] TR Turkey
- [X] TT Trinidad and Tobago
- [X] UA Ukraine
- [ ] UG Uganda
- [X] US United States of America
- [X] UZ Uzbekistan
- [X] VN Viet Nam

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:

- [X] IL Israel
- [ ] .....
- [ ] .....
- [ ] .....

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of _____

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box *If the Supplemental Box is not used, this sheet need not be included in the request.*

Use this box in the following cases:

1. If, in any of the Boxes, the space is insufficient to furnish all the information:	*in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient;*
in particular:	
(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available:	*in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III;*
(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;*
(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America:	*in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;*
(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents:	*in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;*
(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "Continuation" or "Continuation-in-part":	*in such case, write "Continuation of Box No. V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;*
(vi) if there are more than three earlier applications whose priority is claimed:	*in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI.*
2. If the applicant claims, in respect of any designated Office, the benefits of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty:	*in such case, write "Statement Concerning Non-Prejudicial Disclosures or Exceptions to Lack of Novelty" and furnish that statement below.*

IV. AUTRES MANDATAIRES

VAN MALDEREN Joëlle
VAN MALDEREN Michel

Box No. VI PRIORITY CLAIM Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1)			
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☐ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : _____

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA /_____

Earlier search *Fill in where a search (international, international-type or other) by the International Searching Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)*: Number:

Box No. VIII CHECK LIST

This international application contains the following number of sheets:

1. request	5	sheets
2. description	27	sheets
3. claims	7	sheets
4. abstract	1	sheets
5. drawings	5	sheets
Total	45	sheets

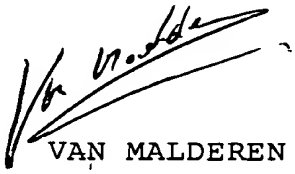
This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☐ copy of general power of attorney
3. ☐ statement explaining lack of signature
4. ☐ priority document(s) *identified in Box No. VI as item(s):*
5. ☒ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify)*:

Figure No. 3 of the drawings (if any) should accompany the abstract when it is published.

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request).



VAN MALDEREN Eric

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1. Date of actual receipt of the purported international application: 23 JUIL 1996 (23-07-1996)
2. Drawings: ☒ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. ☐ Transmittal of search copy delayed until search fee is paid

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Date of receipt of the record copy by the International Bureau:

PROCESS FOR THE PREPARATION OF MALTED CEREALS

Field of the invention.

The present invention is related to an improved process for the preparation of malted cereals, the improved malted cereals obtained and their use, especially in biotechnological processes for the preparation of beverages.

Technological background of the invention.

Cereals such as barley, wheat, rye, corn, oats, rice, millet, triticale, and sorghum are used for the production of beverages. In most cases, they have been subjected to a malting process to take advantage of their increased enzymatic potential.

In traditional malting processes the moisture content of cereals is raised either by immersion(s) and/or spraying(s), and the resulting high moisture content cereal is allowed to germinate. After reaching the proper physiological condition, it is preferably submitted to (a) drying step(s). In what follows the term steeping refers to the increase in moisture level while the term germination is

used in the way it is in plant physiology. The drying operations are referred to as kilning and the term malting involves all operations needed to convert barley (or other cereals) to barley malts(or other cereal malts).

The quality of the malt obtained is, to a large extent, determined by the presence of plant endogenous enzymes generated during the malting process. For instance with cereals like barley used as a raw material for the malt production, the variety, the composition of the microbial flora and the environmental factors, such as agricultural practice, influence the quality of the malt. During cultivation and storage, cereals are contaminated with bacteria and fungi. In the malting plant, neither the air, the water nor the equipment are sterile, and the conditions of humidity, pH and temperature favour the growth of the microbial populations.

The variable cereal quality and the lack of means to make up for deficiencies during the malting process result in variability in malt quality. In many instances, this has to do with an imbalance of specific enzymatic potential and insufficient cell wall degradation. Apart from this, problems with microbial safety can occur. As a consequence of the defects in malt, quality problems occur in the production of beer, such as a poor filtration of the wort.

State of the Art.

During the malting of barley, the microflora develops and the quality of malt and beverages is influenced by the activity of the endogenous micro-organisms.

In analogy with other biotechnological processes, there have been attempts to optimise malt quality aspects by the addition of starter cultures during the malting process

(Boivin, P. & Malanda, M., Influence of Starter Cultures in Malting on the Microflora Development and Malt Quality, EBC, Proceedings of the 24th Congress, pp. 95-102 (1993); Haikara, A. et al., Lactic Starter Cultures in Malting - A Novel Solution to Gushing Problems, European Brewery Convention, Proceedings of the 24th Congress, pp. 163-172 (1993)).

Addition of spores of Geotrichum candidum to the steeping water results in the inhibition of the development of undesirable micro-organisms and in a decrease of the filtration time of wort made of the obtained malt. Treatment with Geotrichum candidum also inhibits the formation of mycotoxins by Fusarium spp.

The influence of Lactobacillus plantarum and Pediococcus pentosaceus has been tested on the microflora during malting and it has been found that these cultures act as natural preservatives as they restrict the growth of Fusarium and prevent gushing.

The international patent application WO94/29430 describes a process for improving the properties of malted cereals wherein starter cultures which comprise moulds, yeasts or bacteria are added prior and/or during malting of said cereals.

The preferred bacteria used are lactic acid producing bacteria such as various Lactobacilli, e.g. Lactobacillus casei, Lactobacillus casei var rhamnosus, Lactobacillus fermentum, Lactobacillus plantarum and Lactobacillus brevis, and bacteria of the genus Pediococcus, e.g. Pediococcus acidilactici.

Preferred moulds are moulds of the genus Aspergillus and Geotrichum, like Geotrichum candidum.

However, malt prepared according to the present invention is of better quality than that prepared according

to WO 94/29430. This is exemplified by higher ß-glucanase and xylanase activities, lower ß-glucan contents in malt and wort and improved European Brewery Convention analytical data.

Aims of the invention.

The present invention aims to provide an improved preparation process for malted cereals and improved malted cereals.

A main aim of the invention is to provide an improved preparation process for malted cereals and improved malted cereals in terms of brewing performances, especially malted cereals having an improved quality in terms of enzymatic potential and microbial safety.

Another aim is to provide a process and improved malted cereals which vary less in quality with the raw material used.

A further aim of the invention is to obtain malted cereals which improve the biotechnological production process of beverages and may improve the properties of the said obtained beverages.

Summary of the invention.

The present invention is related to a process for the preparation of malted cereals, wherein the steeping step includes one or more wetting stages at a temperature between 5 and 30 °C, preferably between 10 and 20 C, until the material has a moisture content between 20 and 60% by weight, preferably between 38 and 47%, wherein after a germination period, between 2 and 7 days, preferably between 3 to 6 days at a temperature between 10 and 30 °C, preferably between 14 and 18 °C, the steeped and germinated cereals are preferably kilned by increasing the temperature to values between 40 and

150 °C, preferably between 45 and 85 °C, until the material has a moisture content between 2 and 15% by weight, preferably between 4 and 7%, and wherein one or more microbial cultures selected from the group consisting of one or more bacteria and/or one or more fungi are added in one or more times, either before or during or after the malting process of said cereals.

This process, thus, allows for a broad flexibility in malting conditions.

Preferably, for the preparation of malted barley, said bacteria are gram positive bacteria or gram negative bacteria, selected from the group consisting of Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp. preferentially Pediococcus halophilus, Pediococcus cerevisiae, Pediococcus damnosus, Pediococcus hemophilus, Pediococcus parvulus, Pediococcus soyae, Lactococcus spp., Lactobacillus spp. preferentially Lactobacillus acidophilus, Lactobacillus amylovorus, Lactobacillus bavaricus, Lactobacillus bifermentans, Lactobacillus brevis var lindneri, Lactobacillus casei var casei, Lactobacillus delbrueckii, Lactobacillus delbrueckii var lactis, Lactobacillus delbrueckii var bulgaricus, Lactobacillus fermenti, Lactobacillus gasserii, Lactobacillus helveticus, Lactobacillus hilgardii, Lactobacillus renterii, Lactobacillus saké, Lactobacillus sativorius, Lactobacillus cremoris, Lactobacillus kefir, Lactobacillus pentoceticus, Lactobacillus cellobiosus, Lactobacillus bruxellensis, Lactobacillus buchnerii, Lactobacillus coryneformis, Lactobacillus confusus, Lactobacillus florentinus, Lactobacillus viridescens, Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter

spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp. preferentially Pseudomonas amylophilia, Pseudomonas aeruginosa, Pseudomonas cocovenenans, Pseudomonas mexicana, Pseudomonas pseudomallei, Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp.

Preferably, for the preparation of malted barley the fungi are selected from the group (genera as described by Ainsworth and Bisby's dictionary of the fungi, 8th edition, 1995, edited by DL Hawksworth, PM Kirk, BC Sutton, and DN Pegler (632 pp) Cab International) consisting of Ascomycota preferentially Dothideales preferentially Mycosphaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp.; Hypocreales preferentially Hypocreceae preferentially Hypocrea spp.; Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae prefentially Hanseniaspora spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp.; Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp.

preferentially Mucor circinelloides, Mucor grisecyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Mucor rouxii, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp. preferentially Rhizopus arrhizus, Rhizopus oligosporus, Rhizopus oryzae preferentially strains ATCC 4858, ATCC 9363, NRRL 1891, NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, Rhizopus formosaensis, Rhizopus chinensis, Rhizopus cohnii, Rhizopus japonicus, Rhizopus nodosus, Rhizopus delemar, Rhizopus acetorinus, Rhizopus chlamydosporus, Rhizopus circinans, Rhizopus javanicus, Rhizopus peka, Rhizopus saito, Rhizopus tritici, Rhizopus niveus, Rhizopus microsporus; Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp. preferentially Monilia candida, Monilia sitophila, Mycoderma spp., Candida spp. preferentially Candida diddensiae, Candida edax, Candida etchellsii, Candida kefir, Candida krisei, Candida lactosa, Candida lambica, Candida melinii, Candida utilis, Candida milleri, Candida mycoderma, Candida parapsilosis, Candida obtux, Candida tropicalis, Candida valida, Candida versatilis, Candida guilliermondii, Rhodotorula spp., Torulopsis spp., Geotrichum spp. preferentially Geotrichum amycelium, Geotrichum armillariae, Geotrichum asteroides, Geotrichum bipunctatum, Geotrichum dulcitum, Geotrichum eriense, Geotrichum fici, Geotrichum flavo-brunneum, Geotrichum fragrans, Geotrichum gracile, Geotrichum heritum, Geotrichum klebaknii, Geotrichum penicillatum, Geotrichum hirtum, Geotrichum pseudocandidum, Geotrichum rectangulatum, Geotrichum suaveolens, Geotrichum vanryiae, Geotrichum loubieri, Geotrichum microsporum, Cladosporium spp.,

Trichoderma spp. preferentially Trichoderma hamatum, Trichoderma harzianum, Trichoderma koningii, Trichoderma pseudokoningii, Trichoderma reesei, Trichoderma virgatum, Trichoderma viride, Oidium spp., Alternaria spp. preferentially Alternaria alternata, Alternaria tenuis, Helminthosporium spp. preferentially Helminthosporium gramineum, Helminthosporium sativum, Helminthosporium teres, Aspergillus spp. as described by R.A. Samson ((1994) in Biotechnological handbooks, Volume 7 : Aspergillus, edited by Smith, J.E. (273 pp), Plenum Press) preferentially Aspergillus ochraseus Group (Thom & Church), Aspergillus nidulans Group (Thom & Church), Aspergillus versicolor Group (Thom & Church), Aspergillus wentii Group (Thom & Raper), Aspergillus candidus Group (Thom & Raper), Aspergillus flavus Group (Raper & Fennell), Aspergillus niger Group (Thom & Church); Penicillum spp. preferentially Penicillum aculeatum, Penicillum citrinum, Penicillum claviforme, Penicillum funiculosum, Penicillum italicum, Penicillum lanoso-viride, Penicillum emersonii, Penicillum lilacinum, Penicillum expansum.

Preferably, for the preparation of malted cereals other than malted barley, especially for the preparation of malted wheat, rye, corn, oats, rice, millet, triticale, and sorghum, said bacteria are gram positive or gram negative bacteria selected from the group consisting of Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp., Lactococcus spp., Lactobacillus spp., Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp., Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp. or a mixture thereof; and said

fungi are fungi selected from the group consisting of : Ascomycota preferentially Dothideales preferentially Mycophaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp., Hypocreales preferentially Hypocreaceae preferentially Hypocrea spp., Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccaromycodaceae preferentially Hanseniaspora spp., Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp., Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Clamydomucor spp., Mucor spp., Rhizopus spp.; Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp., Mycoderma spp., Candida spp., Rhodotorula spp., Torulopsis spp., Geotrichum spp., Cladosporium spp., Trichoderma spp., Oidium spp., Alternaria spp., Helminthosporium spp., Aspergillus spp., Penicillium spp.

According to a preferred embodiment, the preparation process of malted cereals according to the invention comprises the following steps: the steeping step

includes one or more wetting stages or the total time of submersion in water during steeping for physiological reasons does not exceed 30 hours (preferably 10 to 25 hours) or the kilning step includes more than two temperature steps and the microbial cultures which are added, are preferably selected from the group consisting of Rhizopus spp., preferably Rhizopus oryzae such as Rhizopus oryzae strain ATCC9363 and/or Pseudomonas spp., preferably Pseudomonas herbicola.

According to the invention, the malted cereals are selected from the group consisting of barley, wheat, rye, corn, oats, rice, millet, triticale, and sorghum.

In the process according to the invention, the same or different microbial cultures are added in one or more time(s). The microbial cultures used are preferably fungal cultures, preferably spores, and most preferably activated spores. The use of activated spores greatly enhances their contribution to improved malt quality, most likely because of more vigorous growth. The activated spores have one of the following properties: the treated spores are significantly more swollen than their dormant size, more particularly, the size of the spores is increased by a factor preferably between 1.2 and 10 over their dormant size and/or one or more germ tubes per spore are formed. The activated spores are prepared by subjecting them to environmental changes, preferably by one or a combination of the following treatments:

(a) cycles of wetting and/or drying;

(b) addition of appropriate nutritional supplies (such as a nitrogen source, preferably amino acids and/or a carbon source, preferably mono- or disaccharides) or spore elements;

(c) exposure to temperature changes, preferably within a temperature range of 0 to 80 °C;

(d) exposure to changes in pH, preferably within a pH range of 2.0 to 8.0, more preferably between 3.0 and 6.0.

The specialist may easily select precise treatment steps to obtain either swelling of the spores and/or germ tubes as above-mentioned.

The present invention also concerns the malted cereals obtained according to the process of the invention, which present improved European Brewery Convention analysis results. Said improvements may have to do with modification and/or increased hydrolytic enzyme activities. At the same time, a decreased level of toxins, an increased microbial safety by e.g. outcompeting undesirable microbial flora such as Fusarium and/or an increased acceptability compared to the malted cereals according to the state of the art, may be observed.

For instance, the malted cereals according to the invention may have a lower ß-glucan content or a higher ß-glucanase or xylanase activity (as represented in the following examples and figures) than the malted cereals according to the state of the art. This allows for a better processability of the malt in wort and beer production as exemplified by increased rates of filtration.

Another object of the present invention concerns the use of the malted cereals according to the invention for the preparation of beverages.

The invention is also related to these improved beverages.

The improved malted cereals according to the invention could also be used in other biotechnological processes well known by the Man Skilled in the Art, in which

in most cases advantage is taken of their improved quality.

The present invention will be further described in various examples in view of the following drawings.

Brief description of the drawings.

Figure 1 represents the ß-glucanase activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1)

Figure 2 represents the xylanase activity of malted barley obtained according to the preparation process of example 1. (legend: see example 1)

Figure 3 represents the ß-glucanase activity of malted barley obtained according to the preparation process of example 3.(legend: see example 3)

Figure 4 represents the xylanase activity of malted barley obtained according to the preparation process of example 3. (legend: see example 3)

Figure 5 represents the relative increase factor (R.I.F.) for bacterial populations (see text, malt evaluation, example 2) (legend: see example 2)

Example 1.

1. Preparation of microbial cultures

Strain

- S46 : Rhizopus oryzae ATCC 9363

Preparation of the spore suspension

- the strain was grown on PDA (Potato Dextrose Agar, Oxoid) for approximately 10 days at 28 °C;
- the spores were harvested by flooding the cultures with sterile physiological saline (0.9% NaCl) and by rubbing the sporulated mycelium gently with a sterile spatula;

- the spore suspension was washed twice with sterile physiological saline (0.9% NaCl) by centrifugation (5500 rpm, Sorvall type SS-34 ®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl);
- the spore density was determined microscopically using a Thoma counting chamber.

Activation of the spore suspension

- 10^7 spores were transferred into 20 ml of sterile, acidified TSB (Tryptic Soy Broth, Oxoid), pH = 4.0 and incubated in a shaking water bath during 5 to 6 hours at ± 42 °C;
- the activated spores were harvested by centrifugation (3500 rpm, Sorvall type SS-34 ®, for 15 min), washed once with sterile physiological saline (0.9% NaCl) by centrifugation (3500 rpm, Sorvall type SS-34 ®, for 15 min) and resuspended in sterile physiological saline (0.9% NaCl).

2. Barley

- Plaisant - 1994 French harvest

3. Process

Setup

Malts were made by four different malting processes :

- *A1. traditional malting*
  (without inoculation of any spore suspension)
- *B1. malting according to the invention*
  (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)
- *C1. malting process according to the invention*
  (inoculation of the steeped barley with a suspension of

activated spores of Rhizopus oryzae ATCC 9363)

- *D1. malting process according to the invention* (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Steeping

- the steeping was carried out on a 2 kg base with a total water (tap water) to air dry barley ratio of 1.5:1;
- use was made of 2 fermentors (Bioflo III, New Brunswick Scientific), in which perforated plates were placed;
- temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (± 20 °C);
- during the whole steeping period the barley was aerated (4 liter sterile air per minute);
- steeping was carried out by immersion using the following scheme :

	Temperature (°C)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

Addition of the microbial cultures

- ± 460 g of steeped barley was immersed in 0.5 l of tap water which contained no spores (A1), non-activated spores of Rhizopus oryzae ATCC 9363 (B1, according to the invention) or activated spores of Rhizopus oryzae ATCC

9363 (C1, according to the invention); for B1 and C1, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;

- during the steeping, 10^4 activated spores per gram air dry barley were inoculated to the water of the first wet stage (D1);
- the fluid was removed by draining.

Germination

- germination was carried out in a cylindrical container with perforated lids at a temperature of 16-18 °C during 4 days;
- air was supplied by natural diffusion;
- the containers were slowly rotated on an electronically controlled roller system (Cellroll ®, Tecnorama); i.e. every two hours the containers were rolled for 15 min at 1 rpm.

Kilning

- the kilning was carried out in a Joe White malting unit (Australia)

	Air flow (%)	Recirc. Air (%)	Temp. (°C)	Durat. (h)
First kilning stage	25	0	62	3:00
Second kilning stage	25	0	65	2:00
Third kilning stage	25	0	68	2:00
Fourth kilning stage	25	25	73	2:00
Fifth kilning stage	25	50	78	1:00
Sixth kilning stage	25	75	80	2:00
Seventh kilning stage	25	100	83	6:00
Shut down air off				Time-out

4. Methods of analysis and results

Methods for determination and units of moisture, extract, extract difference, color, total protein content, soluble protein content, Kolbach index, pH, diastatic power, according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

Methods for determination and units of turbidity, friability, homogeneity, whole grains, ß-glucan content, according to Analytica-European Brewery Convention (Fourth Edition, 1987, Brauerei und Getränke-Rundschau, supplement published in 1989).

Postcoloration of the wort is determined after boiling the congress wort under reflux at 108 °C during 2 hours.

The viscosity of the congress wort is determined with the Delta-viscosimeter.

For the determination of the filtration volume, the congress wort is filtered over a Schleicher and Schuell 597 1/2 folded filter. The volume (in ml) that is obtained after

1 hour of filtration is the filtration volume of the wort.

Modification is determined with the Calcofluor apparatus (Haffmans) according to the Carlsberg method (Analytica-European Brewery Convention, Fourth Edition, 1987, Brauerei und Getränke-Rundschau).

The ß-glucanase and xylanase activities are determined with the ß-glucazym method ((Megazyme (Austr.) Pty Ltd (April, 1993)) and the xylazym method ((Megazyme (Austr.)Pty Ltd (September 1995)), respectively.

	Traditional malting process (A1)	**Malting process according to the invention (B1)**	**Malting process according to the invention (C1)**	**Malting process according to the invention (D1)**
Moisture	3.9	4.1	3.8	4.3
Extract	80.3	80.4	80.3	79.8
Extract difference	0.8	0.8	0.4	1.1
Color	3.3	3.3	4.1	4.1
Wort turbidity	1.3	1.2	0.7	0.8
Postcoloration	6	6	7.3	7.5
Total protein content	10.1	10.3	10	10.1
Soluble protein content	4.1	4.4	4.8	5.2
Kolbach index	40.6	42.7	48	51.0
Viscosity	1.57	1.52	1.52	1.54
pH	6.05	6.3	5.87	5.79
Diastatic power	345	349	352	419

	Traditional malting process (A1)	Malting process according to the invention (B1)	Malting process according to the invention (C1)	Malting process according to the invention (D1)
Whole grains	0.3	0.3	0.1	ND
Friability	83	82	83.9	ND
Homogeneity	98.5	97.9	98.6	ND
ß-glucan content	122	108	46	<40
Filtration volume	210	265	290	275
Modification	88.2	90.5	93.4	ND
ß-glucanase activity	214	371	683	3856
Xylanase activity	28	34	56	984

ND : not determined

Figures 1 and 2 represent the ß-glucanase and xylanase activity, respectively of the obtained malted barley (A1, B1, CI, D1). These malted barleys are obtained according to a traditional malting process (A1) or according to the above-described malting process of the invention (B1, CI, D1). The ß-glucanase activity was determined with the ß-glucazym method [Megazyme (Austr.) Pty Ltd. (April, 1993)]. Therefore, malt ß-glucanase activity (U/kg) was calculated as 380 x E (590 nm) + 20. The xylanase activity was determined with the endo 1-4-xylazym method [Megazyme (Austr.) Pty Ltd. (September 1995)] Therefore, malt xylanase activity (U/kg) was calculated as (46.8 x E (590nm)+0.9)x 5.

Example 2

1. Preparation of microbial cultures

Strain

- S46 : Rhizopus oryzae ATCC 9363

Preparation of the spore suspension

- as described in example 1

Activation of the spore suspension

- as described in example 1

2. Barley

- Stander - 1995 North American harvest

3. Process

Setup

Malts were made by six different malting processes :

- *A2. traditional malting process*
  (without inoculation of any spore suspension)
- *B2. malting process according to the invention*
  (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)
- *C2. malting process according to the invention*
  (inoculation of the steeped barley during the first wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)
- *D2. malting process according to the invention*
  (inoculation of the steeped barley during the second wet stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)
- *E2. malting process according to the invention*
  (inoculation of the steeped barley during the third wet

stage with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

- *F2. malting process according to the invention* (inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Steeping and addition of the microbial cultures

- the steeping was carried out on a 300 g base with a total water (tap water) to air dry barley ratio of 5:3;
- use was made of 2000 ml flasks;
- a temperature of 18 °C was maintained during the wet stages and during the air rest stages;
- during the whole steeping period the barley was aerated by means of compressed air;
- steeping was carried out by immersion using the following schedule :

	Duration (h)
First wet stage	6:00
First air rest stage	18:00
Second wet stage	5:00
Second air rest stage	19:00
Third wet stage	2:00

- during the steeping, 10^4 activated spores per gram of air dry barley were inoculated to the water of the first wet stage (C2), of the second wet stage (D2) or of the third wet stage (E2) before immersion of the barley;
- the steeped barley was immersed in 0.5 litre of tap water which contained no spores (A2, C2, D2, E2), non-activated (B2) or activated (F2) spores;

- for B2, and F2, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;
- the fluid was removed by draining.

Germination

- as described in example 1

Kilning

- as described in example 1

Malt evaluation

Determination of the increase of the bacterial population

To judge the evolution of the bacterial population during the malting process, a relative increase factor (R.I.F.) was determined by dividing the total bacterial count occurring on the green malt by the total bacterial count occurring on the barley. The total bacterial count was determined after plating appropriate dilutions of an extract of the kernels on Tryptic Soy Agar (Oxoid) supplemented with 100 ppm pimaricine and after incubation at 28 °C for 3 days.

Figure 5 shows the increase of the bacterial population during the malting according to the preparation process of example 2.

Example 3

1. Preparation of microbial cultures

Strain

- S46 : Rhizopus oryzae ATCC 9363

Preparation of the spore suspension

- as described in example 1

Activation of the spore suspension

- as described in example 1

2. Barley

- Plaisant - 1994 French harvest;

3. Process

Setup

Malts were made by three different malting processes :

- *A3. traditional malting*
  (without inoculation of any spore suspension)
- *B3. malting process according to the invention*
  (inoculation of the steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363)
- *C3. malting process according to the invention*
  (inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363)

Steeping

- the steeping was carried out on a 2 kg base air dry barley with a total water (tap water) to air dry barley ratio of 1.5:1;
- the pH of the steeping water was controlled at pH = 5.5 by addition of lactic acid and NaOH;
- a fermentor (Bioflo III, New Brunswick Scientific), in which a perforated plate was placed, was used for steeping;
- temperature was only controlled during the wet stages; during the air rest stages the system was allowed to reach room temperature (ca.20 °C);
- during the whole steeping period the barley was aerated (4 liter sterile air per minute);

- steeping was carried out by immersion using the following schedule :

	Temperature (°C)	Duration (h)
First wet stage	13	6:00
First air rest stage	20	17:00
Second wet stage	14	5:00
Second air rest stage	20	15:30
Third wet stage	16	2:30

Addition of the microbial cultures

- 460 g of steeped barley was immersed in 0.5 l of tap water which contained no spores (A3), non-activated spores of Rhizopus oryzae ATCC 9363 (B3 according to the invention) or activated spores of Rhizopus oryzae ATCC 9363 (C3 according to the invention); for B3 and C3, the steeped barley was inoculated with 10^4 spores per gram of air dry barley;
- the fluid was removed by draining.

Germination

- as described in example 1

Kilning

- as described in example 1

4. Methods of analysis and results

These were as described in example 1 (4. Methods of analysis and results).

See table on next page. In this table :

* A1/3 : Traditional malting process
  B1/3 : Malting process according to the invention
  C1/3 : Malting process according to the invention

	Example 3 pH control of the steeping water (pH = 5.5)			Example 1 No pH control of the steeping water		
	A3	B3	C3	A1	B1	C1
Moisture	3.8	3.6	3.7	3.9	4.1	3.8
Extract	78.9	80.2	80.7	80.3	80.4	80.3
Extract difference	0.6	0.7	0.4	0.8	0.8	0.4
Color	3.2	4.2	4.4	3.3	3.3	4.1
Wort turbidity	1	1	0.8	1.3	1.2	0.7
Postcoloration	5.1	7	7.2	6	6	7.3
Total protein content	10.2	10.1	10	10.1	10.3	10
Soluble protein content	4	4.4	4.8	4.1	4.4	4.8
Kolbach index	39.2	43.6	48	40.6	42.7	48
Viscosity	1.52	1.53	1.52	1.57	1.52	1.52
pH	6.02	5.97	5.91	6.05	6.03	5.87
Diastatic power	348	333	355	345	349	352
Whole grains	0.2	0.2	0.1	0.3	0.3	0.1
Friability	81	81	85	83	82	83.9
Homogeneity	97.6	97.8	98.9	98.5	97.9	98.6
ß-glucan content	190	57	40	122	108	46
Filtration volume	210	215	200	210	265	290
Modification	84.1	85.5	87.4	88.2	90.5	93.4
ß-glucanase activity	202	931	1322	214	371	683
Xylanase activity	43	65	71	28	34	56

PCT/EP96/00077

Figure 3 represents the ß-glucanase activity, measured according to ß-Glucazym method [Megazyme (AUSTR) Pty. Ltd.] of the malted cereals A3, B3 and C3. Malt ß-glucanase activity (U/kg) was calculated as described in example 1. A3 was obtained by the traditional malting process with pH control of the steeping water (pH = 5.5). B3 resulted from the malting process according to the invention with the inoculation of steeped barley with a suspension of non-activated spores of Rhizopus oryzae ATCC 9363 and with pH control of the steeping water (pH = 5.5). C3 was obtained by the malting process according to the invention with the inoculation of the steeped barley with a suspension of activated spores of Rhizopus oryzae ATCC 9363 and with pH control of the steeping water (pH = 5.5).

These results show the increased ß-glucanase activity when the pH of the steeping water is maintained at around 5.5.

Figure 4 gives the corresponding results for xylanase activity. These were measured according to xylazym method, Megazyme ((AUSTR), Pty. Ltd.(September 1995)). Malt xylanase activity was calculated as described in example 1.

Comparison of the ß-glucanase activity obtained according to examples 1 and 3 with the ß-glucanase activity according to the state of the art as described in WO94/29430

In order to compare the improved results regarding ß-glucanase activity by the present invention, we defined the factor μ as follows:

$$\mu = \frac{\text{ß-glucanase activity of the treated malt}}{\text{ß-glucanase activity of the control malt}}$$

This factor was calculated for control malt and malt treated with Rhizopus oryzae ATCC 9363 as described in examples 1 and 3 of the present invention.

It was also calculated for the data described in WO94/29430 (example 1) where Geotrichum candidum was used.

Both as described in WO94/29430, and in the present application, ß-glucanase activity was determined with the beta-glucazyme method [Megazyme (Austr) Pty. Ltd. (April 1993)]. Therefore, malt ß-glucanase activity (U/kg) was calculated as 380 x E(590 nm) + 20 and one unit of activity was defined as the amount of enzyme required to release one micromole of reducing sugar equivalents per minute under the defined above conditions.

Comparison of the results:

State of the art				Invention			
	µ		µ	Ex. 1	µ	Ex. 3	µ
Gc *	1.48	Gc *	1.98	B1/A1	1.73	B3/A3	4.61
				C1/A1	3.19	C3/A3	6.54
				D1/A1	18.02		

*Gc : Geotrichum candidum

The results clearly show that the present invention provides for a more drastic increase in malt ß-glucanase activity than that described earlier (WO 94/29430).

It thus appears that it is possible to obtain malted cereals having a ß-glucanase activity increased by at least a factor 4 compared to the conventional malting process wherein the addition of microbial culture is omitted.

From figure 2 and 4, it also appears that it is possible to obtain malted cereals having a xylanase activity increased by at least a factor 4 compared to conventional

malting process wherein the addition of microbial culture is omitted.

CLAIMS.

1. Process for the preparation of malted cereals, wherein the steeping step includes one or more wetting stages at a temperature between 5 and 30 °C, preferably between 10 and 20 °C, until the material has a moisture content between 20 and 60% by weight, preferably between 38 and 47%, wherein after a germination period between 2 and 7 days, preferably between 3 to 6 days at a temperature between 10 and 30 °C, preferably between 14 and 18 °C, the steeped and germinated cereals are preferably kilned by increasing the temperature to values between 40 and 150 °C until the material has a moisture content between 2 and 15% by weight, and wherein one or more microbial cultures selected from the group consisting of one or more bacteria and/or one or more fungi are added in one or more times either before or during or after the malting process of said cereals.

2. Process according to claim 1, for the preparation of malted barley, wherein the bacteria are gram positive bacteria or gram negative bacteria selected from the group consisting of Micrococcus spp., Streptococcus spp., Leuconostoc spp., Pediococcus spp. preferentially Pediococcus halophilus, Pediococcus cerevisiae, Pediococcus damnosus, Pediococcus hemophilus, Pediococcus parvulus, Pediococcus soyae, Lactococcus spp., Lactobacillus spp. preferentially Lactobacillus acidophilus, Lactobacillus amylovorus, Lactobacillus bavaricus, Lactobacillus bifermentans, Lactobacillus brevis var lindneri, Lactobacillus casei var casei, Lactobacillus delbrueckii, Lactobacillus delbrueckii var lactis, Lactobacillus delbrueckii var bulgaricus, Lactobacillus fermenti, Lactobacillus gasserii, Lactobacillus helveticus, Lactobacillus hilgardii, Lactobacillus renterii,

Lactobacillus saké, Lactobacillus sativorius, Lactobacillus cremoris, Lactobacillus kefir, Lactobacillus pentoceticus, Lactobacillus cellobiosus, Lactobacillus bruxellensis, Lactobacillus buchnerii, Lactobacillus coryneformis, Lactobacillus confusus, Lactobacillus florentinus, Lactobacillus viridescens, Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp. preferentially Pseudomonas amylophilia, Pseudomonas aeruginosa, Pseudomonas cocovenenans, Pseudomonas mexicana, Pseudomonas pseudomallei, Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp.

3. Process according to claim 1, for the preparation of malted barley wherein the fungi are selected from the group (genera as described by Ainsworth and Bisby's dictionary of the fungi, 8th edition, 1995, edited by DL Hawksworth, PM Kirk, BC Sutton, and DN Pegler (632 pp) Cab International) consisting of Ascomycota preferentially Dothideales preferentially Mycosphaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp.; Hypocreales preferentially Hypocreceae preferentially Hypocrea spp.; Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccharomycodaceae

prefentially Hanseniaspora spp.; Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp.; Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Chlamydomucor spp., Mucor spp. preferentially Mucor circinelloides, Mucor grisecyanus, Mucor hiemalis, Mucor indicus, Mucor mucedo, Mucor piriformis, Mucor plumbeus, Mucor praini, Mucor pusillus, Mucor silvaticus, Mucor javanicus, Mucor racemosus, Mucor rouxianus, Mucor rouxii, Mucor aromaticus, Mucor flavus, Mucor miehei, Rhizopus spp. preferentially Rhizopus arrhizus, Rhizopus oligosporus, Rhizopus oryzae preferentially strains ATCC 4858, ATCC 9363, NRRL 1891, NRRL 1472, Rhizopus stolonifer, Rhizopus thailandensis, Rhizopus formosaensis, Rhizopus chinensis, Rhizopus cohnii, Rhizopus japonicus, Rhizopus nodosus, Rhizopus delemar, Rhizopus acetorinus, Rhizopus chlamydosporus, Rhizopus circinans, Rhizopus javanicus, Rhizopus peka, Rhizopus saito, Rhizopus tritici, Rhizopus niveus, Rhizopus microsporus; Mitosporic fungi preferentially Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp. preferentially Monilia candida, Monilia sitophila, Mycoderma spp., Candida spp. preferentially Candida diddensiae, Candida edax, Candida etchellsii, Candida kefir, Candida krisei, Candida lactosa, Candida lambica, Candida melinii, Candida utilis, Candida milleri, Candida mycoderma, Candida parapsilosis, Candida obtux, Candida tropicalis, Candida valida, Candida versatilis, Candida guilliermondii, Rhodotorula spp., Torulopsis spp., Geotrichum spp. preferentially Geotrichum

amycelium, Geotrichum armillariae, Geotrichum asteroides, Geotrichum bipunctatum, Geotrichum dulcitum, Geotrichum eriense, Geotrichum fici, Geotrichum flavo-brunneum, Geotrichum fragrans, Geotrichum gracile, Geotrichum heritum, Geotrichum klebaknii, Geotrichum penicillatum, Geotrichum hirtum, Geotrichum pseudocandidum, Geotrichum rectangulatum, Geotrichum suaveolens, Geotrichum vanryiae, Geotrichum loubieri, Geotrichum microsporum, Cladosporium spp., Trichoderma spp. preferentially Trichoderma hamatum, Trichoderma harzianum, Trichoderma koningii, Trichoderma pseudokoningii, Trichoderma reesei, Trichoderma virgatum, Trichoderma viride, Oidium spp., Alternaria spp. preferentially Alternaria alternata, Alternaria tenuis, Helminthosporium spp. preferentially Helminthosporium gramineum, Helminthosporium sativum, Helminthosporium teres, Aspergillus spp. as described by R.A. Samson ((1994) in Biotechnological handbooks, Volume 7 : Aspergillus, edited by Smith, J.E. (273 pp), Plenum Press) preferentially Aspergillus ochraseus Group (Thom & Church), Aspergillus nidulans Group (Thom & Church), Aspergillus versicolor Group (Thom & Church), Aspergillus wentii Group (Thom & Raper), Aspergillus candidus Group (Thom & Raper), Aspergillus flavus Group (Raper & Fennell), Aspergillus niger Group (Thom & Church), Penicillum spp. preferentially Penicillum aculeatum, Penicillum citrinum, Penicillum claviforme, Penicillum funiculosum, Penicillum italicum, Penicillum lanoso-viride, Penicillum emersonii, Penicillum lilacinum, Penicillum expansum.

4. Process according to claim 1 for the preparation of malted cereals other than malted barley wherein the bacteria are gram positive or gram negative bacteria chosen from the group consisting of Micrococcus spp., Streptococcus

spp., Leuconostoc spp., Pediococcus spp., Lactococcus spp., Lactobacillus spp., Corynebacterium spp., Propionibacterium spp., Bifidobacterium spp., Streptomyces spp., Bacillus spp., Sporolactobacillus spp., Acetobacter spp., Agrobacterium spp., Alcaligenes spp., Pseudomonas spp., Gluconobacter spp., Enterobacter spp., Erwinia spp., Klebsiella spp., Proteus spp.

5. Process according to claim 1 for the preparation of malted cereals other than malted barley wherein the fungi chosen from the group consisting of : Ascomycota preferentially Dothideales preferentially Mycophaerellaceae preferentially Mycosphaerella spp., Venturiaceae preferentially Venturia spp.; Eurotiales preferentially Monascaceae preferentially Monascus spp., Trichocomaceae preferentially Emericilla spp., Euroteum spp., Eupenicillium spp., Neosartorya spp., Talaromyces spp., Hypocreales preferentially Hypocreaceae preferentially Hypocrea spp., Saccharomycetales preferentially Dipodascaceae preferentially Dipodascus spp., Galactomyces spp., Endomycetaceae preferentially Endomyces spp., Metschnikowiaceae preferentially Guilliermondella spp., Saccharomycetaceae preferentially Debaryomyces spp., Dekkera spp., Pichia spp., Kluyveromyces spp., Saccharomyces spp., Torulaspora spp., Zygosaccharomyces spp., Saccaromycodaceae preferentially Hanseniaspora spp., Schizosaccharomycetales preferentially Schizosaccharomycetaceae preferentially Schizosaccharomyces spp.; Sordariales preferentially Chaetomiaceae preferentially Chaetomium spp., Sordariaceae preferentially Neurospora spp., Zygomycota preferentially Mucorales preferentially Mucoraceae preferentially Absidia spp., Amylomyces spp., Rhizomucor spp., Actinomucor spp., Thermomucor spp., Clamydomucor spp., Mucor spp., Rhizopus spp.; Mitosporic fungi preferentially

Aureobasidium spp., Acremonium spp., Cercospora spp., Epicoccum spp., Monilia spp., Mycoderma spp., Candida spp., Rhodotorula spp., Torulopsis spp., Geotrichum spp., Cladosporium spp., Trichoderma spp., Oidium spp., Alternaria spp., Helminthosporium spp., Aspergillus spp., Penicillium spp.

6. Process according to any of the preceding claims, wherein the total time of submersion in water during steeping for physiological reasons does not exceed 30 hours, preferentially takes 10 to 25 hours, or wherein the kilning includes more than two temperature steps and wherein the microbial culture comprises Rhizopus spp. and/or Pseudomonas spp.

7. Process according to the claim 6, wherein the Rhizopus sp. is preferably a Rhizopus oryzae such as a Rhizopus oryzae strain ATCC 9363.

8. Process according to the claim 6, wherein the Pseudomonas sp. is preferably a Pseudomonas herbicola.

9. Process according to any of the preceding claims, wherein the microbial spores used are activated by one or a combination of the following treatments:

- (a) cycles of wetting and/or drying,
- (b) addition of nutritional supplies or addition of spore elements,
- (c) exposure to temperatures changes, preferably within a range of 0 to 80 °C,
- (d) exposure to changes in pH, preferably within a pH range of 2.0 to 8.0, more preferably between 3.0 and 6.0, to obtain spores significantly more swollen than their dormant size, more particularly, the size of the spores is increased by a factor preferably between 1.2 and 10 over their dormant size and/or spores with one or more

germ tubes per spore.

10. Process according to any of the preceding claims, wherein the pH during the steeping step is adjusted to a value between 4.0 and 6.0.

11. Process according to any of the preceding claims, wherein nutrients and/or additives are added prior to and/or during the malting process.

12. Malted barley characterized by a β-glucanase activity increased by at least a factor 4 and a xylanase activity increased by at least a factor 4, compared to the conventional malting process of any available barley.

13. Malted barley, wherein the β-glucanase activity is higher than 700 units/kg. and the xylanase activity is higher than 250 units/kg.

14. Malted barley according to claim 12 or 13 obtained by the process of any of the claims 1 to 11.

15. Malted barley according to any of the claims 12 to 14, characterized in that they present an improved modification and/or an increased hydrolytic enzyme activity, a decreased level of toxins and/or increased microbial safety or increased acceptability.

16. Use of the malted cereals according to any of the claims 12 to 15, or obtained by the process of any of the claims 1 to 11 for the preparation of beverages.

ABSTRACT

PROCESS FOR THE PREPARATION OF MALTED CEREALS

Process for the preparation of malted cereals, wherein the steeping step includes one or more wetting stages at a temperature between 5 and 30 °C, preferably between 10 and 20 °C, until the material has a moisture content between 20 and 60% by weight, preferably between 38 and 47%, wherein after a germination period between 2 and 7 days, preferably between 3 to 6 days at a temperature between 10 and 30 °C, preferably between 14 and 18 °C, the steeped and germinated cereals are preferably kilned by increasing the temperature to values between 40 and 150 °C until the material has a moisture content between 2 and 15% by weight, and wherein one or more microbial cultures selected from the group consisting of one or more bacteria and/or one or more fungi are added in one or more times either before or during or after the malting process of said cereals.

(Figure 3).

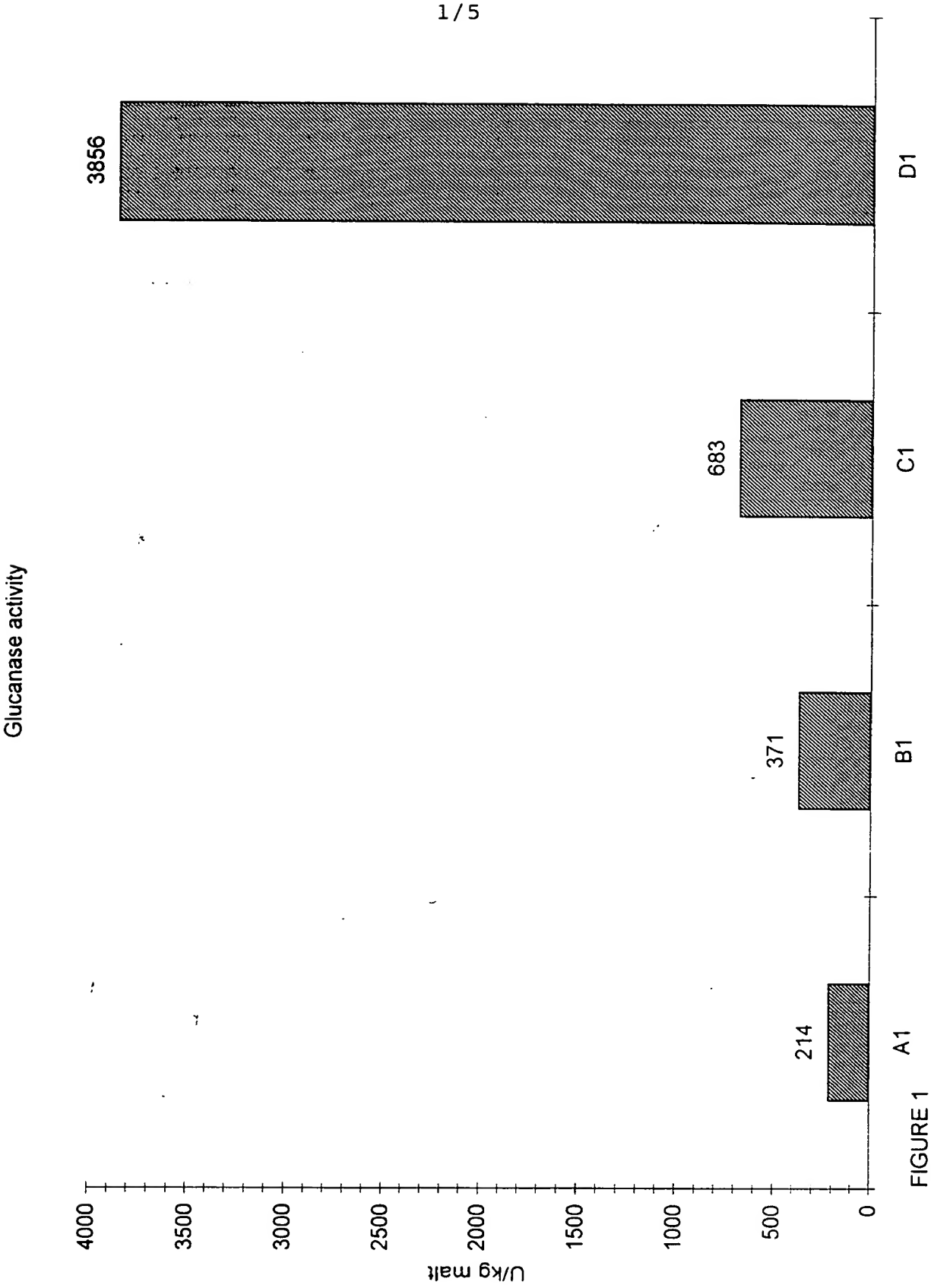
Glucanase activity
U/kg malt
4000
3500
3000
2500
2000
1500
1000
500
0
214
371
683
3856
A1
B1
C1
D1

FIGURE 1

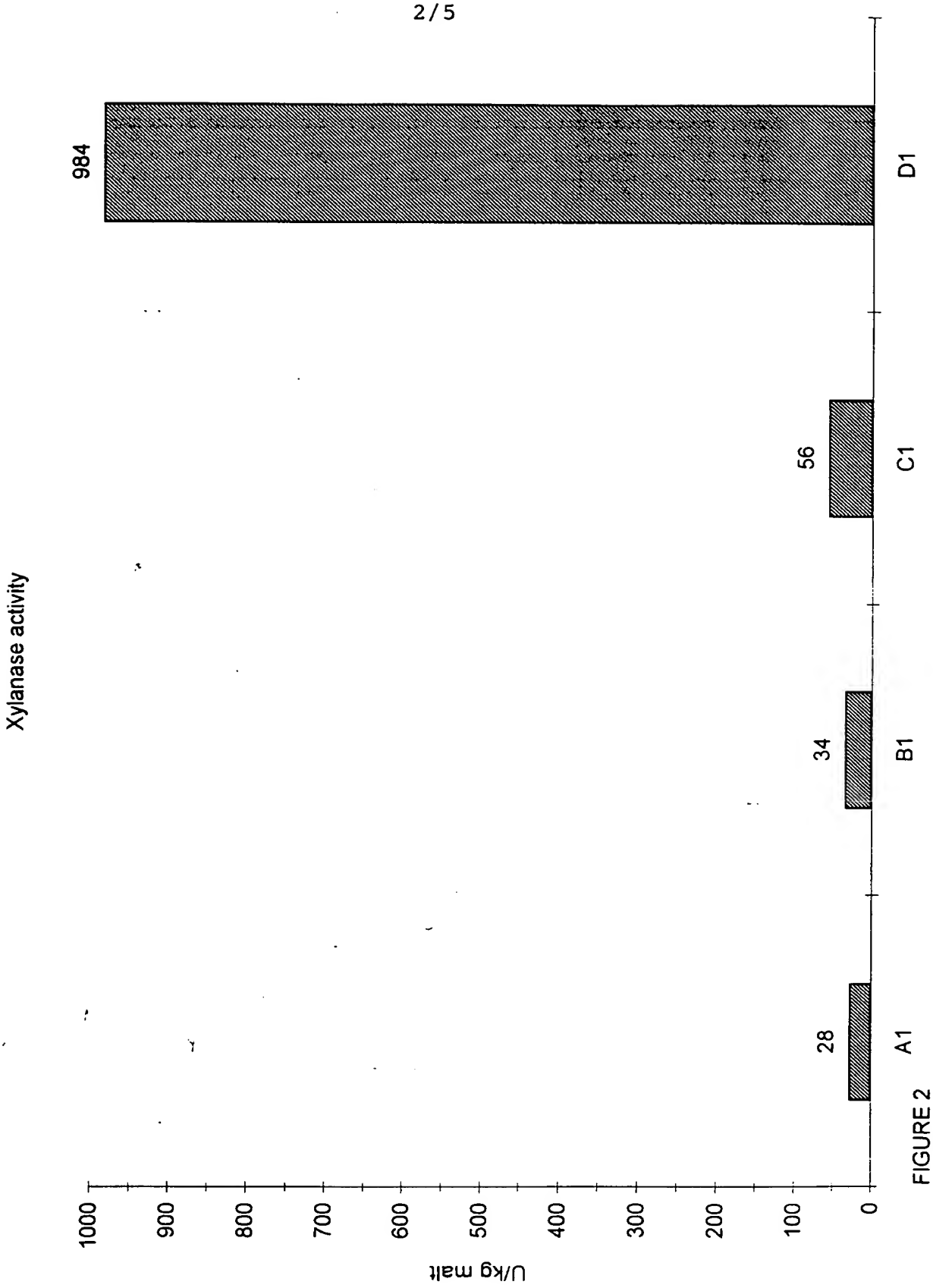
Xylanase activity
U/kg malt
1000
900
800
700
600
500
400
300
200
100
0
984
56
34
28
D1
C1
B1
A1

FIGURE 2

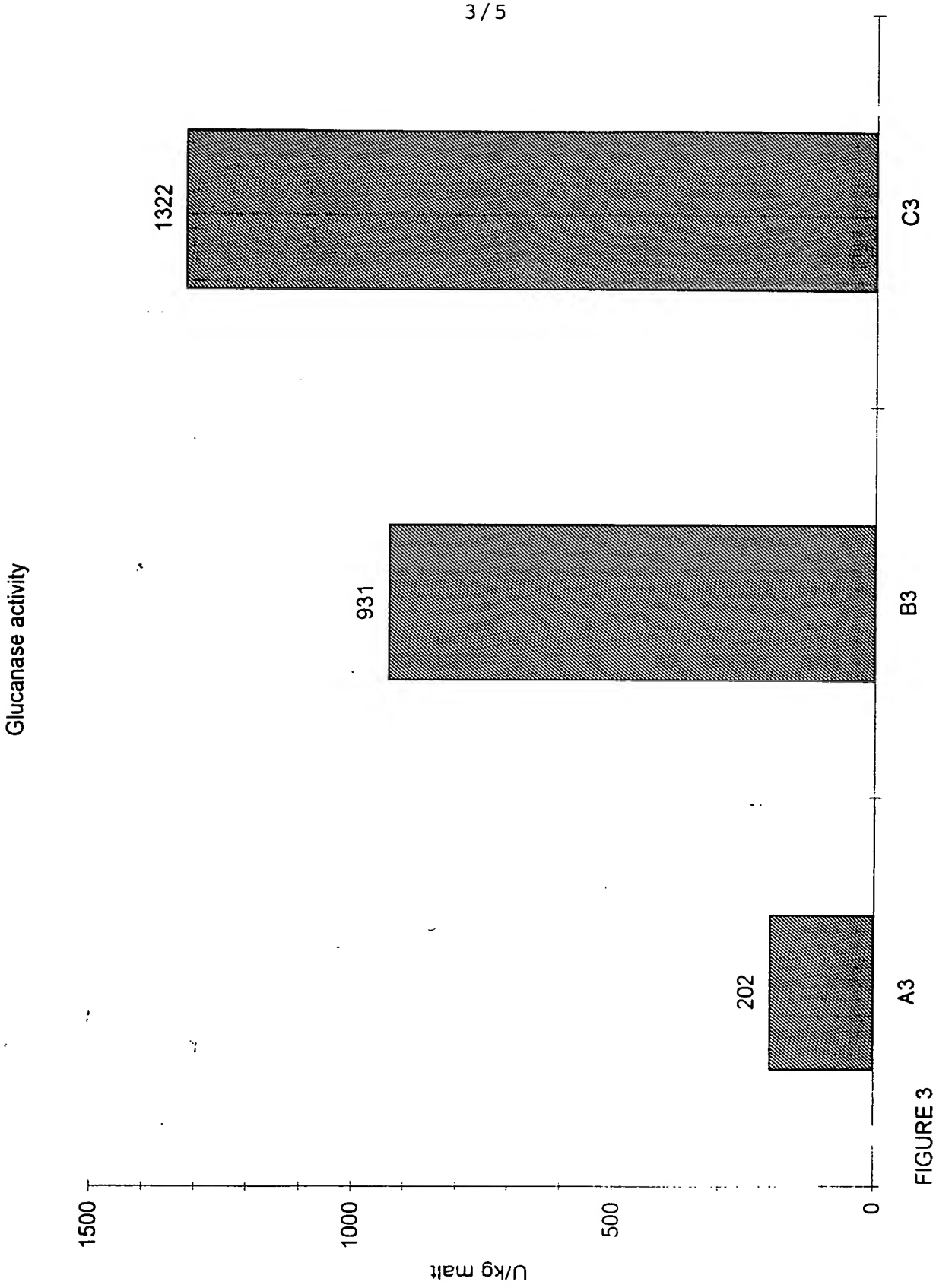
Glucanase activity
U/kg malt
1500
1000
500
0
1322
931
202
C3
B3
A3

FIGURE 3

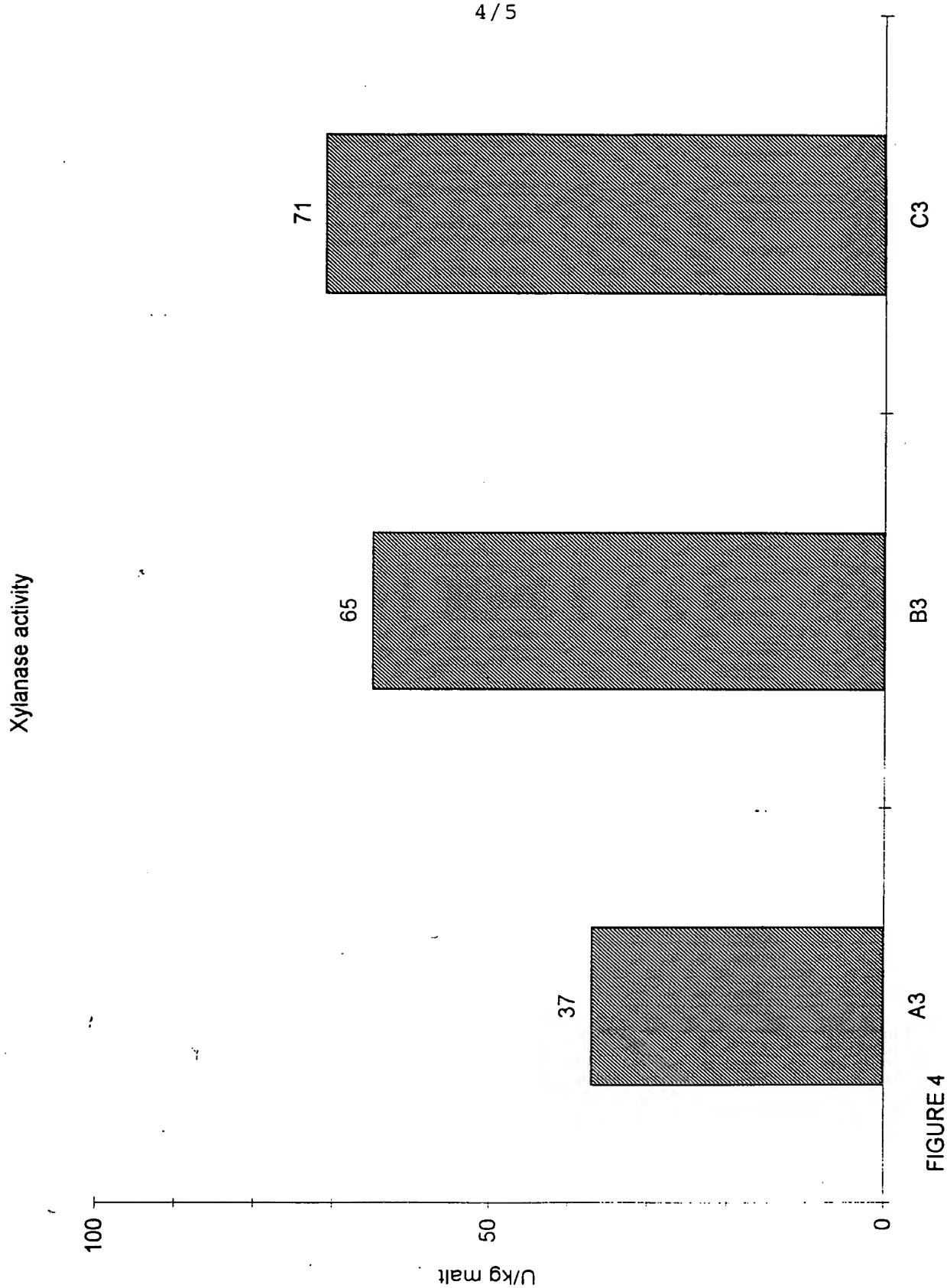

FIGURE 4

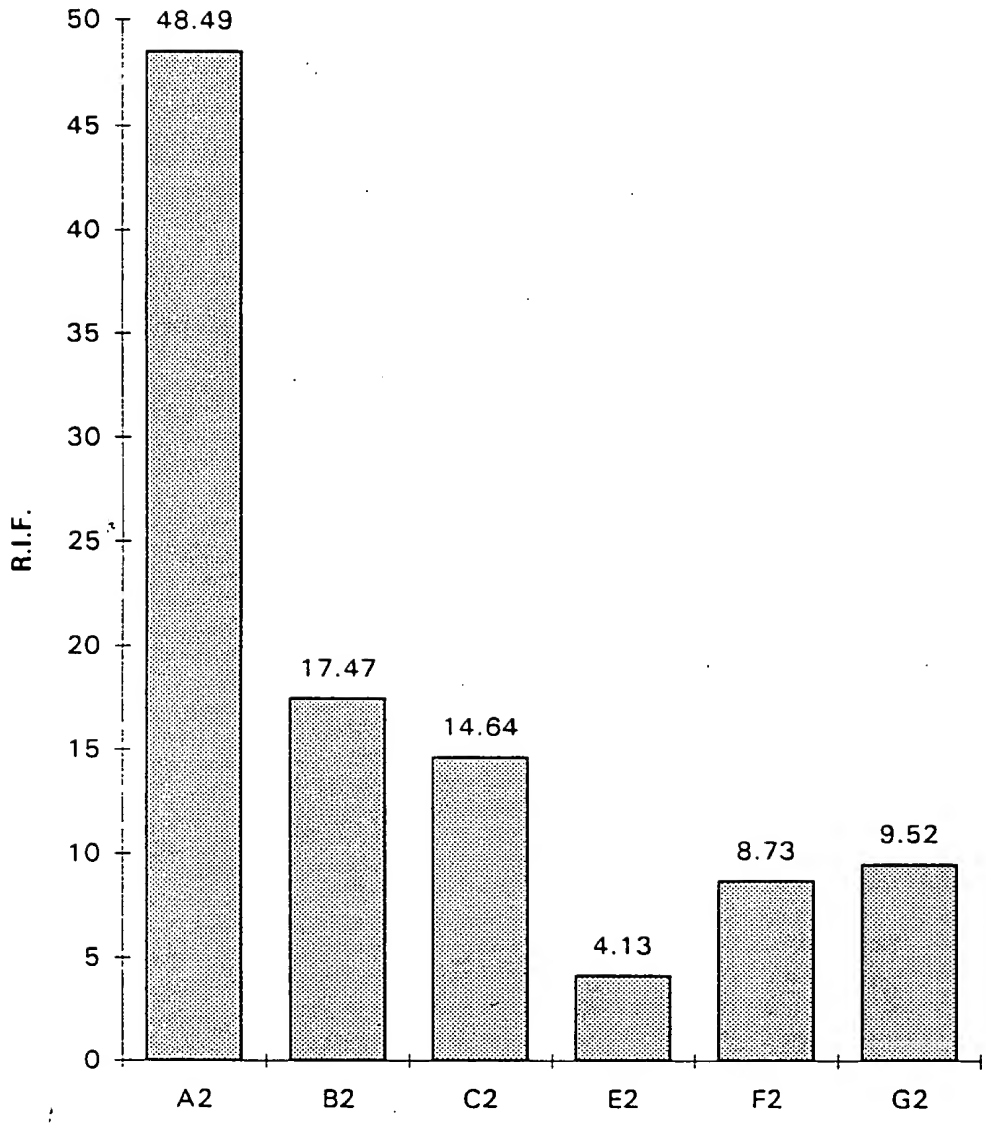
R.I.F.
50
45
40
35
30
25
20
15
10
5
0
48.49
17.47
14.64
4.13
8.73
9.52
A2
B2
C2
E2
F2
G2

FIGURE 5